On the Orientation of Minute Objects for the Microtome.

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With 7 Text-figures.

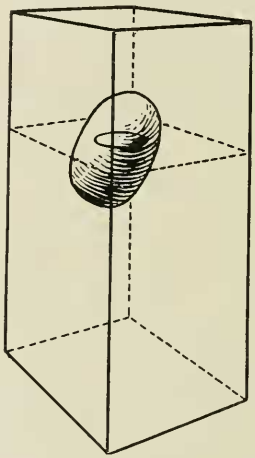
For some time past the work I have been engaged upon has necessitated the sectioning of large numbers of minute larvæ, whose small size and lack of obvious symmetry or external "landmarks" made them extraordinarily difficult things to prepare for the microtome, and in no degree amenable to the ordinary methods of embedding. None of the various devices that are current will give, in my experience, anything approaching exact orientation—upon which often depends the very possibility of interpreting the sections when they are cut; and this is because in none of them are the essential operations conducted at leisure, at the ordinary temperature, and with the object plainly seen under the microscope. A method which combines the satisfaction of these requirements with the advantages of double-embedding in collodion and wax is given below. By its use I have been able to obtain precise orientation of such small objects as the early larval stages of Amphioxus, Ciona and Cucumaria, and of the cleavage stages of Echinus and Ascidiella.

I am persuaded that the usefulness of such a technique to workers whose researches are not (as mine are) interrupted by the war will excuse its present publication unfortified by

a context of material results. Modifications will doubtless suggest themselves to the reader, and indeed the ensuing description is designedly only an outline.

The principle of the method is to enclose the specimen to be cut in a mass of collodion large enough to be seen and handled in paraffin wax and with a definite geometrical form, the long axis of which is at right angles to the plane of intended section (Text-fig. 1). This primary object may be

TEXT-FIG. 1.



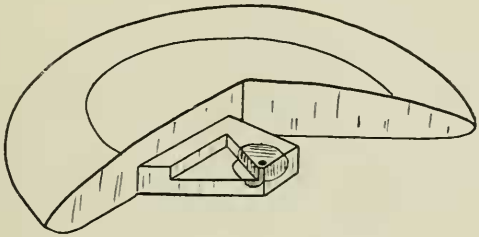
attained in one or other of the following two ways, according as a greater or a less degree of accuracy is necessary in the orientation. The first furnishes a convenient means of handling small objects in all cases where the greatest nicety is not required.

(i) The fixed and hardened specimens, lightly stained, are transferred from "absolute" alcohol to oil of cloves, and thence to a thin syrup made by mixing a thick alcohol-ether solution of collodion[1] with an equal volume of oil of cloves. Such a syrup has a high index of refraction (i.e. is a clearing reagent), and can be thickened to any convenient consistency

[1] Grübler's or Schering's "celloidin" was used.

by allowing the volatile constituents to evaporate. After remaining in it for twenty-four hours, in a covered capsule, the specimens are picked up singly in a pipette, and allowed to fall, each surrounded by a drop of syrup, into chloroform, in which they are left till they are quite clear and have sunk to the bottom of the receptacle. Each glassy globule of hardened collodion is then embedded for from twenty to thirty minutes in paraffin wax (melting-point 52°–56° C.) in the thermostat, transferred to molten wax in a glycerine-coated watch-glass, and cooled quickly in the ordinary way. The cast so obtained is now pared away on one side till the globule stands

Text-fig. 2.



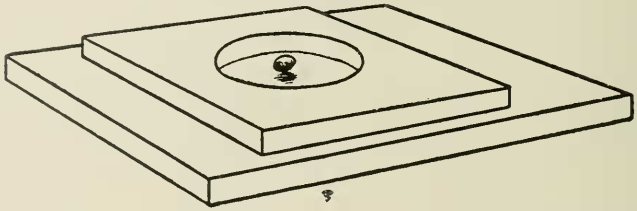
in a salient solid angle of wax, care being taken to retain a sufficient mass of wax to serve as a handle during the next operation, which involves cutting the collodion itself. In doing this it is important to shave away only a little at a time, so that the passage of the knife shall deform the small piece removed and cause no compression of the parent block —a matter of importance when the embedded object is a delicate one. With a thin, sharp knife (a safety-razor blade is excellent for the purpose), and preferably under a binocular dissecting microscope, the collodion is cut away until the object comes plainly into view (Text-fig. 2) and is made to occupy the end of a slender rod, the axis of the specimen either coinciding with that of the rod, or being at right angles to it, according as the sections are to be transverse or longitudinal. The little rod is then separated from its parent mass, embedded for about a minute in hard wax, and

prepared for the microtome in the usual way, the collodion rod being set vertical upon the carrier.

It is possible to obtain in this way, with a considerable degree of precision, transverse or longitudinal sections of objects of which only one main axis can be determined in the external view, or in which no discrimination between longitudinal planes is desired; but where the plane of section is required to be in relation to the planes of internal organs, visible only in the cleared object, under the microscope, a greater refinement of means is necessary.

(ii) A microscope slide, cut down to two-thirds of its original length, is coated on one side with a thin, continuous

Text-fig. 3.



film of paraffin wax by drawing a drop of molten wax over its heated surface with the edge of another slide, as in making a blood-film. A square of glass with a circular hole in it is coated thinly with wax and cemented on the prepared slide by means of heat; or if such squares are not available, four slips of glass, cut from a thin slide, may be used to the same end. In this way a shallow cell is obtained, lined throughout with wax, and to this the specimen is transferred with sufficient collodion syrup to fill the cell, the amount being adjusted by means of a fine pipette so that the surface of the liquid is a plane (Text-fig. 3). The depth of the cell must be suited to the diameter of the object, but it should not be less than a millimetre, or the resulting rod of collodion will lack rigidity, on which the success of the method depends.

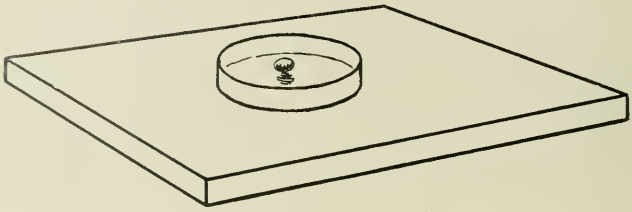
The slide is now placed in a shallow Petri dish upon the stage of a vertically set microscope under the lowest power

objective that will serve to show the structures to be orientated, and the specimen is manipulated with the point of a fine needle till the plane of intended section is vertical. Generally there is no great difficulty in achieving this, because it matters not at all what aspect of the object is upward so long as one line in the chosen plane is vertical, and in the case of the most minute objects this initial orientation is obtained by tilting the slide or moving the medium with the point of a needle. But in refractory cases it may be necessary to prop the object with a fragment of collodion-impregnated tissue, or, better, with a flake of egg-albumen that has been fixed, stained, and embedded in the same way as the specimen itself.

When the specimen has been so arranged enough xylene is poured into the Petri dish to cover the surface of the preparation, which is then left untouched on the stage of the microscope for fifteen to twenty minutes, after which the collodion is sufficiently "set" to prevent rotation of the specimen. Xylene is used here in preference to chloroform or cedar oil as being a lighter liquid and less liable to disturb the surface of the collodion. As soon as this is set the preparation is transferred to a second dish and covered with cedar oil, in which it is left overnight. This completes the clearing and hardening of the collodion, and, by dissolving or softening the paraffin wax, makes it possible to remove the glass square. The object is now contained in a thin plate of collodion, with its plane of section normal to the surfaces of the plate, which in practice always remains attached to the microscope slide (Text-fig. 4). It remains to fix one other spatial direction. The excess of cedar oil having been drained off the slide, and the surface, except in the neighbourhood of the collodion wafer, cleaned with alcohol and dried, the preparation is placed upon the mechanical stage of a microscope and the specimen brought into the centre of the field, the slide being rotated until the plane of intended section is at right angles to the lateral movement of the mechanical stage. The position of the specimen in the field of a low-power objective

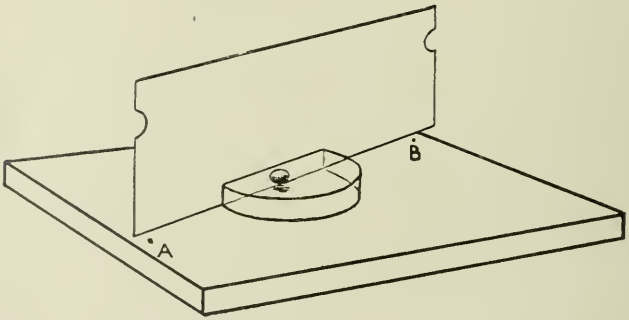
is now noted (by means of an ocular micrometer or the like) and the stage shifted laterally till an area of cleaned glass slide fills the field. A mark is made with a fine brush and Indian ink on that part of the slide which occupies the

Text-fig. 4.



position in the field already noted, and the stage is moved in the reverse direction and the operation repeated on the other side of the collodion wafer. Two points (Text-figs. 5 and 6 A and B) are thus obtained which accurately fix the axis of

Text-fig. 5.

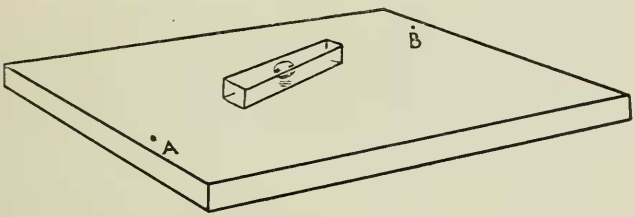


the desired rod of collodion, and using these as guides this rod can now be cut with a straight-edged knife. It is detached from the slide and prepared for the microtome as before.

It may be asked: Why not pour the collodion into a waxed rectangular gutter and orientate the object once and for all

with the plane of intended section at right angles to the length of the gutter—i. e. to the axis of the resulting rod of collodion? The answer is that the operation of placing a body in a viscous liquid so that one of its planes shall be at right angles to a given straight line (the axis of the gutter) is many times more difficult than that of placing it with the given plane at right angles to a plane (the upper surface of the collodion). Added to this is the difficulty of working with needles in a narrow gutter. A method, brought to my notice by the late C. H. Martin, in which a streak of collodion syrup containing the object is drawn across a waxed slide by means of a pipette, orientation being then effected with

TEXT-FIG. 6.



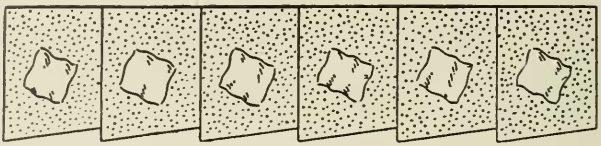
reference to the direction of the streak, is open to these same and other objections. Manipulation is made difficult by surface-tension effects; the curvature of the surface of the highly refractile liquid distorts the image of the object, and the resulting rod of collodion is not a symmetrical one.

In conclusion, a few general considerations concerning the cutting of double-embedded objects may be added.

The greatest bugbear of the method is undoubtedly the difficulty, which arises from time to time, of flattening the sections upon the slide. Folding of the collodion under the shear of the microtome knife will always occur to some extent, but so long as this folding is approximately equivalent to that in the surrounding wax it will disappear automatically in the ordinary process of flattening. It is differential folding, or puckering, of the collodion that must be reckoned

with. This is due to the difference of elasticity between the two media : the wax is compressed and telescoped under the shear of the knife, while the collodion retains its area undiminished, and is thrown into puckers in order to accommodate its perimeter to the reduced total area of the section. Such puckers (Text-fig. 7) are unaffected by flattening the surrounding wax—unless, indeed, it is actually melted—and prevention should be relied upon rather than cure. The microtome knife should be sharp, and inclined as little as possible to the plane of section, its cutting movement slow. A machine, such as Jung's, in which the knife is directly actuated by the hand, is better than one in which a system of

Text-fig. 7.



levers, or the like, intervenes. Paraffin of high melting-point (about 56° C.) gives the best results, and the thickness of the collodion syrup should be adjusted to the hardness of the wax by means of two or three blank experiments. The collodion rod must be made as narrow as is consistent with the safety of the contained object.

If, with these precautions taken, there are signs of puckering in the early stages of cutting a block, a satisfactory series can often be obtained by increasing the thickness of the sections by a micron. Where, however, this cannot be done, or is ineffective, and the collodion is still puckered after the wax has been flattened, the following method should be tried. The excess of water used in flattening the sections is run off the slide, and the lengths of ribbon moved into the position they are to occupy. A cigarette-paper, wet with absolute alcohol, is now cautiously brought down on them, and over this is laid a piece of stout filter-paper. The whole

system is held firmly down on the bench by placing the thumb of the left hand over the part of the slide where the label will be put, and the pad of the right thumb or fore-finger is then drawn several times with firm, even pressure over the covered slide, from left to right. On removing the filter-paper, the cigarette-paper quickly dries and separates from the surface of the slide, leaving the sections firmly adhering. This seemingly heroic method is perfectly safe when applied to sections in a tough, elastic medium like collodion. It is quite inapplicable to ordinary wax sections.